BIOREMEDIATION OF GASOLINE CONTAMINATED SOIL BY BACTERIAL CONSORTIUM AMENDED WITH POULTRY LITTER, COIR PITH AND RHAMNOLIPID BIOSURFACTANT

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ABSTRACT

The aim of the present study was to find methods for enhancing rates of hydrocarbon biodegradation in gasoline contaminated soil by ex-situ bioremediation. Red soil (RS) was treated with gasoline spilled soil (GS) from a gasoline station and different combinations of amendments were prepared using (i) mixed bacterial consortium (MC) (ii) poultry litter (PL) (iii) coir pith (CP) and (iv) rhamnolipid biosurfactant (BS) produced by Pseudomonas sp. DS10-129. The study was conducted for a period of 90 days during which bacterial growth, hydrocarbon degradation and growth parameters of Phaseolus aureus RoxB including seed germination, chlorophyll content, shoot and root length were measured. Approximately 67% and 78% of the hydrocarbons were effectively degraded within 60 days in soil samples amended with RS+ GS+MC+PL+CP+BS at 0.1% and 1% respectively. Maximum percentage of seed germination, shoot length, root length and chlorophyll content in P. aureus were recorded after 60 days in the above amendments. Further incubation to 90 days did not exhibit significant improvements. Statistical analysis using Analysis of Variance (ANOVA) and Duncan's Multiple Range test (DMRT) revealed that the level of amendments, incubation time and combination of amendments significantly influenced bacterial growth, hydrocarbon degradation, seed germination and chlorophyll content at a 1% probability level. All tested additives MC, PL, CP and Rhamnolipid biosurfactant had significant positive effects on the bioremediation of gasoline spilled soils.

Key words: Bioremediation, Gasoline-spilled soil, amendments, mixed consortium, poultry litter, coir pith, biosurfactant, *Phaseolus aureus* RoxB, plant growth parameters.

INTRODUCTION

Advances in science and technology since the industrial revolution has increasingly enabled human to exploit natural resources. However, this has generated unprecedented disturbances in global elemental cycles (Trabalka & Reichle, 1986). The relatively sudden introduction of xenobiotic chemicals, or the massive relocation of natural material to different environmental compartments can often overwhelm the self cleaning capacity of recipient ecosystems and thus result in the accumulation of pollutants to problematic or even harmful levels. In addition to minimising the impact of future incidents by controlling contaminant input, pollutant decay should be accelerated to remedy existing problems.

Oil contamination with petroleum hydrocarbons has caused critical environmental and health defects and increasing attention has been paid for developing and implementing innovative technology for cleaning up this contamination (Yeung *et al*., 1997). During accidental spills, action can be taken to remove or remediate or recover the contaminant immediately, whereas in gasoline stations, the spills due to leakage may be small but continuous and prolonged. Because of its persistence, chances for contamination of the groundwater are more likely. Bioremediation methods are currently receiving favourable publicity as promising environmentally friendly treatment technologies for the remediation of hydrocarbons (Desai & Banat, 1997).

Bioremediation can be described as the conversion of chemical compounds by viable organisms, especially microorganisms with novel catabolic functions derived through selections or by the introduction of genes encoding such functions into energy, cell mass and harmless biological waste products. For petroleum hydrocarbons, these biological waste products are primarily CO_2, water and methane (Walter *et al*., 1997). As no single microbial species is capable of degrading all components of crude oil, complete oil degradation requires simultaneous action of different microbial populations. One of the factors that

limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms (Providenti *et al*., 1995). Generally petroleum hydrocarbon compounds bind to soil components and are difficult to remove or degrade. Biosurfactants can emulsify hydrocarbons, thus enhancing their water solubility, decreasing surface tension and increasing the displacement of oily substances from soil particles (Banat *et al.*, 1995a,b; Banat *et al*., 2000).

Following oil pollution, nutrients are rapidly assimilated by the soil microorganisms thus depleting the nutrient reserves. Therefore, apart from the environmental problems caused by oil pollution, the agronomic and economic aspects are significant (Jobson *et al*., 1974; Kuhn *et al.*, 1998). The objective of using amendments is to augment the native fertility status of such soils and to enhance the rate of oil degradation, thus minimising the contamination of scarce groundwater sources and to improve crop production (Amadi, 1990). The addition of organic waste material such as poultry litter and coir pith to the soil facilitates aeration through small pores and increases the water holding capacity of the soil, thus enhancing bioremediation (Jobson et.al., 1974; Amadi, 1992). This study was designed to test the use of mixed consortium (MC), poultry litter (PL) coir pith (CP) and biosurfactant (BS) on gasoline spilled soil (GS) and study the bioremediation potential by observing bacterial growth, oil degradation and growth parameters of green gram (*Phaseolus aureus* RoxB).

METHODS

Sample preparation

The red soil was collected from the Bharathiar University campus. Gasoline contaminated soil samples collected from 10 different gasoline stations in Coimbatore City were mixed thoroughly and used for the preparation of amendments. Crude oil degrading mixed bacterial consortium containing five strains (*Micrococcus* sp. GS2-22, *Bacillus* sp. DS6-86, *Corynebacterium* sp. GS5-66, *Flavobacterium* sp. DS5-73,

Pseudomonas sp. DS10-129) previously isolated on hydrocarbon containing medium were inoculated in 200 ml of nutrient broth and kept in a shaker for 24 h at room temperature. Members of the mixed consortium were selected depending on the efficiency of crude oil degradation (data not shown). For the preparation of amendments, the poultry litter was collected from a Poultry farm; air-dried and sieved (less than 0.5mm). Decomposed coir pith used in this study was available locally in India for soil conditioning. Rhamnolipid biosurfactant was produced by *Pseudomonas* sp. DS10-129 grown on hydrocarbon containing medium and broth extracted with Chloroform-methanol mixture according to the method described by Rocha *et al*. (1992).

Amendments / supplements

One hundred grams of red soil (RS) was used to prepare the amendments. For the treatment 10g of GS was taken and mixed thoroughly with red soil. To find out the role of indigenous microbial populations present in soil, controls were set up with no amendments. Other amendments containing the mixed microbial consortium (MC) and the other additions (PL, CP, BS) were set up to test the effects of these additives at two different concentrations (0.1% and 1%) (Table 1). The treatments were incubated at room temperature (28^{o}C). Triplicate set of experimental pot were analysed at 1, 15, 30, 60 and 90 days to enumerate total heterotrophic bacterial counts, percentage of oil degradation and ability to support growth of green gram (seed germination, root and shoot length and chlorophyll content) and the mean values were computed.

Enumeration of bacteria at regular intervals

Total heterotrophic bacteria (THB) were enumerated in all the treatments by using pour plate technique on plate count agar (HI-MEDIA, Mumbai, India) which also allowed growth of all members of the added MC.

Hydrocarbon estimation

The total hydrocarbons in the treatments were determined spectrophotometrically following the method of Odu *et al.* (1985). Soil samples from different treatments were mixed with equal volume of toluene to extract hydrocarbons from the soil. The extracted hydrocarbons were detected spectrophotometrically at 420 nm. A standard curve prepared using known concentrations of gasoline was used to estimate the amount of hydrocarbons in the soil samples. Degradation was estimated as the difference between the initial and final concentrations of total hydrocarbons. This method of determination was selected as no gas chromatographic facilities were available at the time.

Growth study of green gram *Phaseolus aureus* (RoxB)

The seeds of green gram were procured from the Pulses Breeding section of Tamil Nadu Agricultural University, India. The seeds were soaked in distilled water for 5min and floating seeds were removed. Viable seeds of the same size were taken and surface sterilised with 0.1% $HgCl_2$ solution for 2-3 minutes (Hartmann *et al*. 1997) and washed with distilled water thoroughly. The experimental soil samples were set up in pots; 10 seeds were placed into each pot at 2cm depth and all pots were watered regularly. The treated seeds were allowed to germinate and germination percentage was assessed on the 5th day of experiment. At the 10th day of the plantation, the shoot length and root length were measured, mean length calculated and the chlorophyll content estimated colorimetrically as described by Sadasivam & Manickam (1996).

Statistical analysis

The experiment was set up as a factorial design consisting of gasoline contaminated soil **x** six treatments; 1) RS; 2) RS+GS; 3) RS+GS+MC; 4) RS+GS+MC+PL; 5) RS+GS+MC+PL+CP; 6)RS+GS+MC+PL+CP+BS **x** five time periods (1, 15, 30, 60 &

90 days) **x** three replicates per treatment. Statistical analysis was carried out using Analysis of Variance (ANOVA). Mean of the various treatments were tested for level of significance at 1% and 5% probability by Duncan's multiple range test (DMRT) (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

The bacterial counts in the untreated control soils varied from approximately 1.1 to 1.3 x 10^5 CFU/g (Fig 1). The addition of the gasoline contaminated soil led to an increase in values to approximately 4.0 x 10^5 CFU/g. The addition of the mixed consortium (MC) of hydrocarbon degrading microorganisms to an *ex-situ* bioremediation lead to a steady increase in the total heterotrophic bacterial counts from approximately 4.6 x 10^5 CFU/g on the first day to approximately 4.2 x 10^6 CFU/g on day 60. All other additives had greater initial microbial populations and also exhibited an increase in numbers up to day 60. On the 90th day, bacterial population decreased in all treatments.

On the 60^{th} day, the population was maximum in RS+GS+MC+PL+CP+BS amended soil indicating the role of nutrients in the enhancement of bacterial population. The control treatment of red and gasoline spilled soil mixture showed no significant increase in the bacterial populations between day one to day 90 at 1% probability level. Increasing numbers with time in all the other treatments indicated possible limitation in soil for nutrients available in PL, PL+CP, PL+CP+BS. The results are similar to the findings of Gian & Jianmei (1996) on gasoline contaminated soil amended with poultry litter. While comparing the concentrations of amendments, maximum degradation was observed in the amendments added at the higher concentration (1%) which may be due to the increased availability of nutrients. Similar observations were reported when amending oil contaminated soil with poultry manure (Amadi & Ue Bari, 1992).

The plating technique was effective in enumerating the total heterotrophic bacterial population, as well as our oil degrading bacterial strains. We monitored bacterial numbers beyond 90 days (data not shown). There was no significant variation in the bacterial population in all the amendments at 1% probability level. Walter *et al*. (1997) reported similar observations with oil contaminated soil under field condition while testing anionic surfactant guanidinium cocoate amended with mixed consortium and vermiculite.

In amendments carried out at high and low concentrations, maximum degradation was observed on the 60th day of treatment (Fig 2). There was no significant increase at day 90. The hydrocarbon degradation was maximum when all supplements were added to the contaminated soil, up to 67% in 0.1% amendments and 78% in 1% amendments. With the addition of each amendment the hydrocarbon degradation increased from approximately 2.0% in the control with no amendments to 36.7, 40.5, 59.0 and 67.0% when supplementing with 0.1% of each MC, MC+PL, MC+PL+CP, and MC+PL+CP+BS, respectively. Supplementing with higher concentration (1.0%) of the above resulted in an increased degradation from approximately 2.0% to 39.0, 42.8, 61.7 and 77.3%, respectively.

Based on our results it appears that addition of biosurfactant to soils contaminated with gasoline was effective in increasing hydrocarbon loss. Grouping and analysis of the study with initial hydrocarbon concentration revealed that the combination of the amendments (RS+GS+MC+PL+CP+BS) was most effective on soils containing petroleum hydrocarbon contamination. Soil amended with low concentration of organic amendments showed less decrease in hydrocarbon degradation. This result may be a specific effect of RS+GS+MC+PL+CP+BS on soil contaminated with gasoline, where hydrocarbon may be less tightly sorbed to the soil particles than in soil containing relatively low concentration of the contaminant. Alternatively it is possible that the

lower concentrations of hydrocarbon consisted of more recalcitrant compounds or the compounds could have been more tightly sorbed on to the soil particles.

Spectrophotometric analysis was used for measuring hydrocarbon degradation due to its simplicity and reasonable efficiency within the concentrations we used. The results were consistent and sensitive enough for our determinations. The addition of the mixed consortium to an *ex-situ* bioremediation may have increased the number of hydrocarbon degrading bacteria, yet it did not appear to affect the amount of hydrocarbon degraded. This may be due to the low hydrocarbon concentrations in our treatments. This observation is in general agreement with the earlier reports regarding the use of bioaugmentation, which were best employed in situations of very high or very low levels of contamination (Huessmann, 1994). Furthermore, the soil treated with the mixed consortium only lost substantially less hydrocarbons than the soils containing all the other additives. The organic amendments supplied might have increased the bacterial population (indigenous and seeded) which enhanced the degradation of hydrocarbons. Further the surfactant applied might have played a role in emulsifying the hydrocarbon which may have been readily available for degradation by the bacterial population.

The plant growth study showed that germination efficiency of the *Phaseolus aureus* RoxB seeds in the uncontaminated soil RS was generally above 90% (in all but one treatment) (Table 2). Germination decreased to approximately 20% in soils containing gasoline and increased with each additive and with time to a maximum after 60-90 days incubated soil (Table 2). The percentage of seed germination increased from 20 to 80% in low concentration (0.1%) amendments and 20 to 90% in higher concentration (1%) amendments. This may be due to the effect of amendments on oil degradation and possible release of toxic metabolites during degradation that could be adsorbed by the organic

compounds present in poultry litter and coir pith. This in turn may have lead to the reduction of the growth inhibiting compounds released in the soil.

The shoot length in the controls without amendments varied between 12.1 to 13.0 cm (Table 3). The addition of gasoline soil (GS) reduced the shoot length to a range of 7.2 to 7.8cm. The addition of MC to the gasoline-contaminated soil did not result in any significant recovery in the shoot length (1% probability level) probably due to the presence of some toxic metabolites. Further amendments PL, CP, BS resulted in an increase in the shoot length with time to values similar or slightly higher than the controls. Maximum shoot length recorded were 12.3cm and 15.3cm when all amendments were added to soil (RS+GS+MC+PL+CP+BS) at 0.1% and 1.0% concentrations, respectively.

Similar patterns of effects were recorded for root length (Table 4). Maximum root length recorded at 0.1% and 1.0% amendments were 8.3 and 9.4cm in the 60 and 90 days remediated soils. These values are slightly higher than those for the control RS, which ranged between 7.3 and 7.9cm.

Chlorophyll content also exhibited similar trend when compared to the shoot and root length responses (Table 5). Maximum chlorophyll content recorded in 0.1% and 1.0% amendments were 1.17 and 1.27 mg/g, respectively. However, an enhancement of plant growth parameters and chlorophyll content with 60 days remediated soil is an indication of the positive effect of soil amendments with MC, MC+PL, MC+PL+CP and MC+PL+CP+BS.

In general, hydrocarbon contamination reduced both seed germination and plant growth because hydrocarbons could coat plant roots influencing water and nutrient absorption (Kuhn *et al*., 1998). Hydrocarbon molecules can penetrate into plant tissues and damage the cell membranes causing leakage of cell contents and block intercellular spaces reducing metabolite transport and respiration rate (Xu & Johnson, 1995).

However, the severity of the effects of hydrocarbons on plant growth varies with the constituents and amount of the hydrocarbons and the plant species involved. In this experiment the reduction of the plant growth for green gram was stable. This means that it could survive or even perform better than barley in relatively higher concentration of hydrocarbon contaminated soils (Baker, 1970). This has potential benefit for reclamation of hydrocarbon-contaminated soils since leguminous species of plant could fix nitrogen and establish a mantle of vegetation rapidly.

All the results were statistically analysed using ANOVA and DMRT procedures to determine significant parameters. The results presented in Table 6 revealed that all the above parameters were highly influenced by single factors (concentration (C), amendments (A), number of days (D) treated); two factor combinations (C x A, A x D and C x D) and three factor combination (C x A x D) at 1% probability level. However the two factor combination C x D was not significant at 1% or 5% probability levels for seed germination, shoot length and root length. Moreover at 5% probability level, C x A x D was not significant for shoot length and root length, but significant for hydrocarbon degradation.

CONCLUSION

Bioremediation of oil pollution is an acceleration of the natural process of oil degradation and hence a natural treatment to the problem. Since microorganisms require nitrogen, phosphorus and other mineral nutrients for incorporation into biomass, the availability of these nutrients within the area of hydrocarbon degradation is usually limiting. Our results have shown that gasoline spilled soil amended with MC+PL+CP+BS exhibited efficient oil degradation. Since individual bacterial cultures can metabolise only a limited range of hydrocarbon substrates, mixed bacterial consortium with broad enzymatic capacities may be more efficient in remediating

gasoline-spilled soils. The addition of rhamnolipid biosurfactant however further enhanced bioremediation of gasoline spilled soil. This is likely due to better solubilisation of hydrocarbons prior to microbial degradation. Both poultry litter and coir pith are potential sources of nutrients for microbial activity. Statistical analyses using ANOVA and DMRT also showed that concentration, amendment and days of treatment at different factorial designs (C, A, D, CxA, CxD, AxD and CxAxD) were significant at 1% probability level for hydrocarbon degradation. Hence bioremediation of gasoline contaminated soil can be achieved by treating with MC, PL, CP and rhamnolipid BS at 1% concentration for 60 days and successfully cultivated with green gram.

ACKNOWLEDGEMENT

KSM Rahman wishes to thank the Council of Scientific and Industrial Research, New Delhi, India for the award of Senior Research Fellowship and financial support. Thanks also to Environment and Heritage Service, DOE, for FRDF financial support under the N.Ireland Single Programme (Ref. WM47/99).

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Table 1. Preparation of various amendments of soil

S. No.	Amendment	*Concentration (0.1%)**						*Concentration (1%)**					
		RS	GS	MC	PL	CP	BS	RS	GS	MC	PL	CP	BS
1.	RS	100						100					
2.	RS+GS	100	10					100	10				
3.	RS+GS+MC	100	10	0.1				100	10	1			
4.	RS+GS+MC+PL	100	10	0.1	0.1			100	10	1	1		
5.	RS+GS+MC+PL+CP	100	10	0.1	0.1	0.1		100	10	1	1	1	
6.	RS+GS+MC+PL+CP+BS	100	10	0.1	0.1	0.1	0.1	100	10	1	1	1	1

RS – Red soil GS – Gasoline spilled soil MC – Mixed consortium

PL – Poultry litter CP – Coir pith BS – Biosurfactant solution

* Units for RS, GS, PL, CP are in grams and for MC and PL in ml

Table 2. Germination of *Phaseolus aureus* Rox B seeds in control and gasoline spilled soil treated with different amendments for a period of up to 90 days.

S. No.	Treatments / Days	Seed germination (%)									
		Concentration(0.1%)						***Concentration(1%)***			
		1	15	30	60	90		1	15	30	60	90
1	RS	95a ± 2.8[@]	80a ± 11.5	90a ± 2.8	95a ± 2.8	97a ± 1.5		95a ± 2.8	93a ± 1.1	94a ± 1.1	97a ± 1.1	92a ± 1.1
2.	RS+GS	20e ± 1.1	20f ± 1.7	20f ± 1.1	30e ± 1.1	30e ± 1.7		20d ± 1.7	20f ± 2.8	20f ± 1.7	20d ± 1.1	30d ± 3.4
3.	RS+GS+MC	20e ± 1.7	30e ± 2.8	30e ± 4.6	40d ± 1.7	40d ± 3.4		20d ± 1.1	30e ± 1.7	40e ± 2.3	50c ± 2.3	50c ± 1.1
4.	RS+GS+MC+PL	30d ± 2.8	40d ± 4.0	40d ± 1.1	60c ± 2.8	60c ± 1.1		40c ± 2.8	40d ± 1.7	50d ± 3.4	70b ± 4.0	70b ± 2.8
5.	RS+GS+MC+PL+CP	40c ± 1.7	50c ± 2.8	60c ± 1.7	60c ± 1.1	60c ± 2.3		40c ± 1.1	60c ± 2.8	70c ± 3.4	70b ± 2.8	70b ± 1.7
6.	RS+GS+MC+PL+CP+BS	50b ± 1.1	70b ± 5.7	70b ± 1.7	80b ± 2.8	80b ± 4.0		50b ± 1.7	70b ± 1.7	80b ± 4.6	90a ± 2.8	90a ± 1.1

RS – Red soil GS – Gasoline spilled soil MC – Mixed consortium

PL – Poultry litter CP – Coir pith BS – Biosurfactant solution

a, b, c, d, e = Arithmetic means with the same letter are not significantly different from each other at 5% probability level by Duncan's Multiple Range Test (DMRT).

@ = Standard Error

Table 3. Shoot length of 10days old seedlings of *Phaseolus aureus* Rox B seeds in control and gasoline spilled soil treated with different amendments for a period of up to 90 days.

S. No.	Treatments / Days	Shoot length (cm)									
		Concentration(0.1%)						***Concentration(1%)***			
		1	15	30	60	90	1	15	30	60	90
1	RS	12.1a ± 0.05[@]	12.3a ± 0.17	12.6a ± 0.34	12.7a ± 0.40	12.93a ± 0.23	12.4a ± 0.23	13.0a ± 0.28	12.83a ± 0.577	12.9b ± 0.23	12.7b ± 0.40
2.	RS+GS	7.2d ± 0.11	7.4d ± 0.23	7.4d ± 0.23	7.7c ± 0.40	7.8d ± 0.17	7.2d ± 0.11	7.5e ± 0.28	7.6d ± 0.34	7.7d ± 0.11	7.7d ± 0.40
3.	RS+GS+MC	7.2d ± 0.23	7d ± 0.28	6.7d ± 0.40	6.5d ± 0.28	6.3e ± 0.17	7.3d ± 0.17	7e ± 0.28	6.6e ± 0.34	6.4e ± 0.23	6.33e ± 0.34
4.	RS+GS+MC+PL	8.4c ± 0.23	8.7c ± 0.40	9.1c ± 0.05	9.4b ± 0.23	9.1c ± 0.05	8.7c ± 0.40	9.3d ± 0.17	9.7c ± 0.40	10.7c ± 0.40	10.6e ± 0.34
5.	RS+GS+MC+PL+CP	9.1bc ± 0.23	10.2b ± 0.11	11.4b ± 0.23	11.9a ± 0.51	11.93b ± 0.23	9.7b ± 0.40	10.2c ± 0.11	11.4b ± 0.23	12.6b ± 0.34	12.5b ± 0.28
6.	RS+GS+MC+PL+CP+BS	9.3b ± 0.17	10.5b ± 0.28	10.9b ± 0.51	12.1a ± 0.05	12.3ab ± 0.17	10.4b ± 0.23	11.3b ± 0.17	13.1a ± 0.05	15.3a ± 0.17	15a ± 0.57

RS – Red soil GS – Gasoline spilled soil MC – Mixed consortium

PL – Poultry litter CP – Coir pith BS – Biosurfactant solution

a, b, c, d, e = Arithmetic means with the same letter are not significantly different from each other at 5% probability level by Duncan's Multiple Range Test (DMRT).

@ = Standard Error

Table 4. Root length of 10days old seedlings of *Phaseolus aureus* Rox B seeds in control and gasoline spilled soil treated with different amendments for a period of up to 90 days.

S. No.	Treatments / Days	Root length (cm)									
		Concentration(0.1%)						***Concentration(1%)***			
		1	15	30	60	90	1	15	30	60	90
1	RS	7.3a ± 0.11[@]	7.7a ± 0.11	7.4a ± 0.23	7.6b ± 0.05	7.9ab ± 0.23	7.2a ± 0.11	7.4a ± 0.23	7.4b ± 0.11	7.5bc ± 0.17	7.7b ± 0.11
2.	RS+GS	4.2c ± 0.11	4.47cd ± 0.11	4.7c ± 0.40	4.7d ± 0.11	4.9d ± 0.23	4.1d ± 0.05	4.4d ± 0.23	4.5d ± 0.28	4.7d ± 0.11	4.77d ± 0.34
3.	RS+GS+MC	4.3c ± 0.17	4d ± 0.11	4d ± 0.23	3.8e ± 0.17	3.8e ± 0.23	4.2d ± 0.11	3.8d ± 0.46	3.7e ±0.40	3.4e ± 0.23	3.4e ± 0.28
4.	RS+GS+MC+PL	4.7bc ± 0.40	4.9c ± 0.11	5.3c ± 0.17	6.1c ± 0.05	6e ± 0.28	5.3c ± 0.17	5.7c ± 0.40	6.2c ± 0.11	6.9c ± 0.23	6.9c ± 0.28
5.	RS+GS+MC+PL+CP	5.2b ± 0.11	5.8b ± 0.46	6.5b ± 0.28	7.3b ± 0.17	7.3b ± 0.28	5.9bc ± 0.23	6.6b ±0.34	7.1b ± 0.05	7.9b ± 0.23	7.77b ± 0.28
6.	RS+GS+MC+PL+CP+BS	5.2b ± 0.11	5.9b ± 0.23	7.3a ± 0.17	8.3a ± 0.17	8.3a ± 0.11	6.3b ± 0.17	7.2ab ± 0.11	8.5a ± 0.28	9.7a ± 0.11	9.4a ± 0.23

RS – Red soil GS – Gasoline spilled soil MC – Mixed consortium

PL – Poultry litter CP – Coir pith BS – Biosurfactant solution

a, b, c, d, e = Arithmetic means with the same letter are not significantly different from each other at 5% probability level by Duncan's Multiple Range Test (DMRT).

@ = Standard Error

Table 5. Chlorophyll content of 10days old seedlings of *Phaseolus aureus* Rox B seeds in control and gasoline spilled soil treated with different amendments for a period of up to 90 days.

S. No.	Treatments / Days	Chlorophyll (mg/g)									
		Concentration(0.1%)						*Concentration(1%)*			
		1	15	30	60	90		1	15	30	60	90
1	RS	1.20a ±0.057[@]	1.23a ±0.017	1.27a ±0.011	1.27a ±0.040	1.24a ±0.023		1.21a ±0.005	1.25a ±0.028	1.27a ±0.040	1.27a ±0.028	1.26a ±0.034
2.	RS+GS	0.70d ±0.057	0.73d ±0.017	0.75d ±0.028	0.75d ±0.028	0.70d ±0.057		0.74d ±0.023	0.75d ±0.028	0.77d ±0.040	0.78d ±0.046	0.78d ±0.017
3.	RS+GS+MC	0.72d ±0.011	0.71d ±0.005	0.70d ±0.057	0.68d ±0.017	0.68d ±0.028		0.75d ±0.023	0.73d ±0.017	0.67e ±0.040	0.63e ±0.017	0.64e ±0.037
4.	RS+GS+MC+PL	0.77cd ±0.040	0.79d ±0.026	0.87c ±0.040	0.91c ±0.005	0.90c ±0.028		0.81cd ±0.005	0.87c ±0.04	0.94c ±0.023	0.98c ±0.046	0.95c ±0.028
5.	RS+GS+MC+PL+CP	0.84c ±0.023	0.89c ±0.023	0.93c ±0.017	0.99c ±0.011	0.97c ±0.011		0.89c ±0.023	0.92c ±0.011	0.97c ±0.040	1.07b ±0.040	1.04b ±0.023
6.	RS+GS+MC+PL+CP+BS	0.97b ±0.040	1.07b ±0.011	1.10b ±0.028	1.17b ±0.011	1.15b ±0.028		1.04b ±0.011	1.13b ±0.017	1.17b ±0.040	1.27a ±0.040	1.24 ±0.023

RS – Red soil GS – Gasoline spilled soil MC – Mixed consortium

PL – Poultry litter CP – Coir pith BS – Biosurfactant solution

a, b, c, d, e = Arithmetic means with the same letter are not significantly different from each other at 5% probability level by Duncan's Multiple Range Test (DMRT).

@ = Standard Error

Table 6. Significance level for the different parameters tested within our treatments computed by Duncan's Multiple Range Test (DMRT)

Parameter	Bacteria (x 10^4 CFU/g)			Hydrocarbon degradation (%)			Seed germination (%)			Shoot length (cm)			Root length (cm)			Chlorophyll content (mg/g)		
Factorial Effect	SE	CD	SL	SE	CD	SL	SE	CD	SL	SE	CD	SL	SE	CD	SL	SE	CD	SL
Concentration (C)	18.26	35.79	**	0.32	0.63	**	0.75	1.47	**	0.07	0.14	**	0.06	0.11	**	0.079	0.156	**
Amendment (A)	28.87	56.59	**	0.50	0.99	**	1.19	2.33	**	0.11	0.23	**	0.09	0.18	**	0.012	0.025	**
Days (D)	31.63	61.99	**	0.55	1.09	**	1.30	2.56	**	0.13	0.25	**	0.10	0.20	**	0.013	0.027	**
C x A	44.73	87.67	**	0.78	1.54	**	1.84	3.62	**	0.18	0.36	**	0.14	0.28	**	0.019	0.038	**
C x D	40.83	80.03	**	0.71	1.40	**	1.68	3.30	ns	0.16	0.33	ns	0.13	0.26	ns	0.017	0.035	**
A x D	70.28	138.6	**	1.24	2.44	**	2.92	5.72	**	0.29	0.57	**	0.23	0.45	**	0.030	0.060	**
C x A x D	100.0	196.0	**	1.76	3.46	*	4.13	8.09	*	0.41	0.80	ns	0.32	0.64	ns	0.043	0.085	**

SE - Standard Error

CD -Cumulative Difference

SL - Significant level

* Significant at 5% probability level

** Significant at 1% probability level

ns - not significant at 1% or 5% probability levels

FIGURES LEGENDS

Fig 1 Bacterial growth during various treatments at regular intervals in gasoline spilled soil. The figure title A is results of the treatment with 0.1 % amendments and title B is results of the treatment with 1.0 % amendments.

Fig 2. Hydrocarbon degradation during various treatments at regular intervals in gasoline spilled soil. The figure title A is results of the treatment with 0.1 % amendments and title B is results of the treatment with 1.0 % amendments.